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(54) Abstract Title

Polynucleotides and polypeptides of the G-protein coupled receptor AXOR87

(57) Polynucleotides and polypeptides from the purinergic-like G-protein coupled receptor AXOR87 and methods for producing such molecules are disclosed. Also described are antibodies specific to the AXOR87 polypeptide and methods of screening compounds to identify those that stimulate or inhibit the function or level of the polypeptide.

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Figure 1: Relative Expression of AXOR87 in Human Tissues

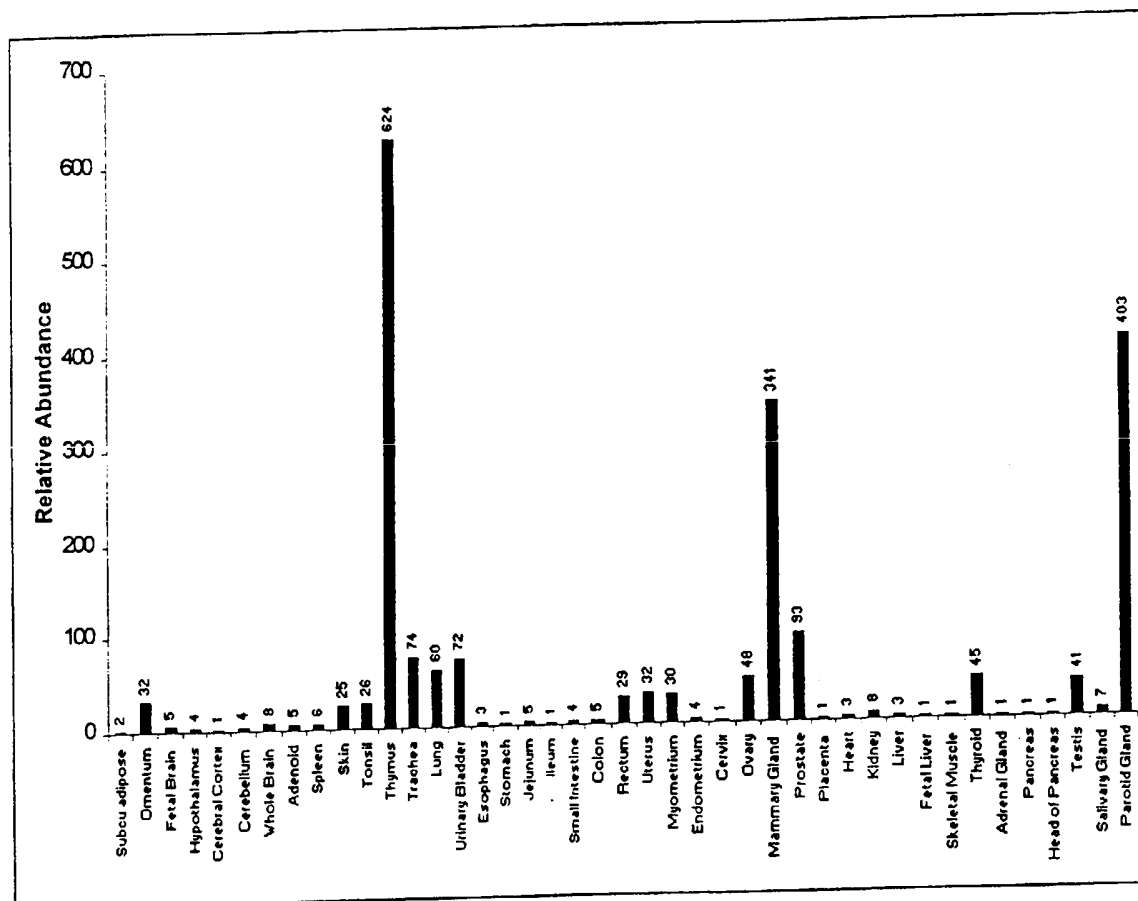


Figure 2: Relative Expression of AXOR87 in Normal and Tumor Tissues

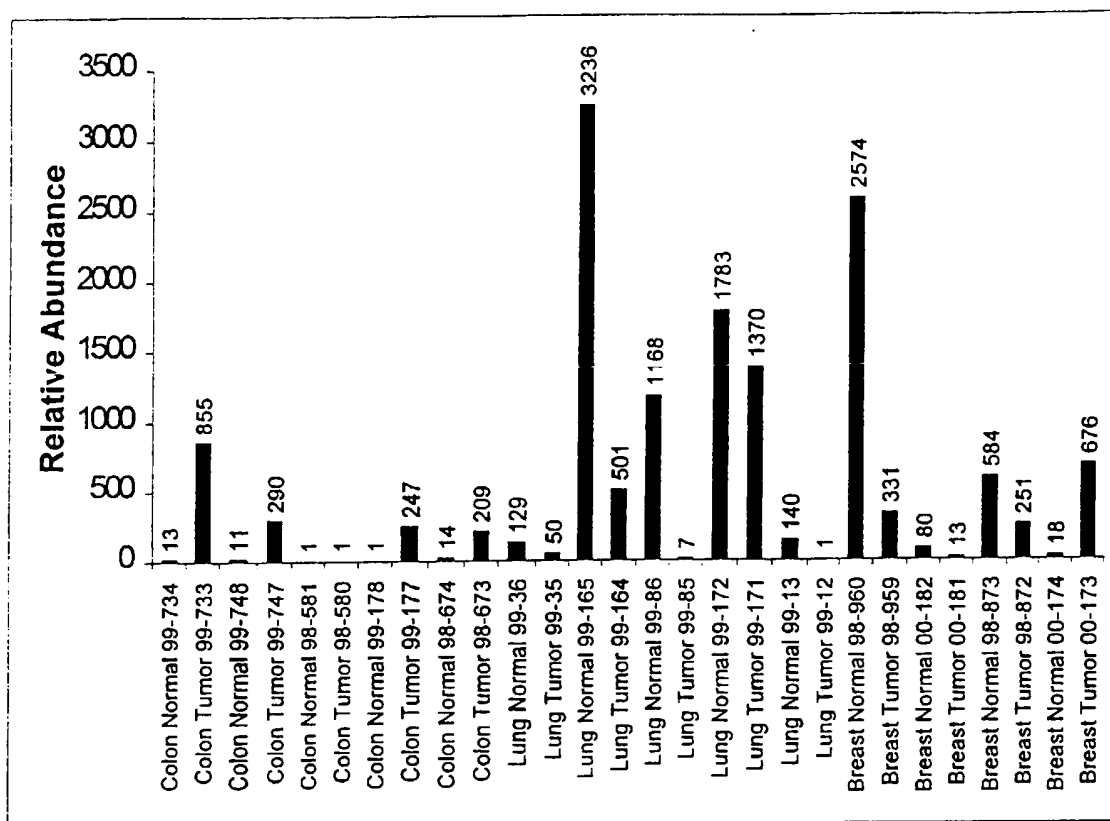
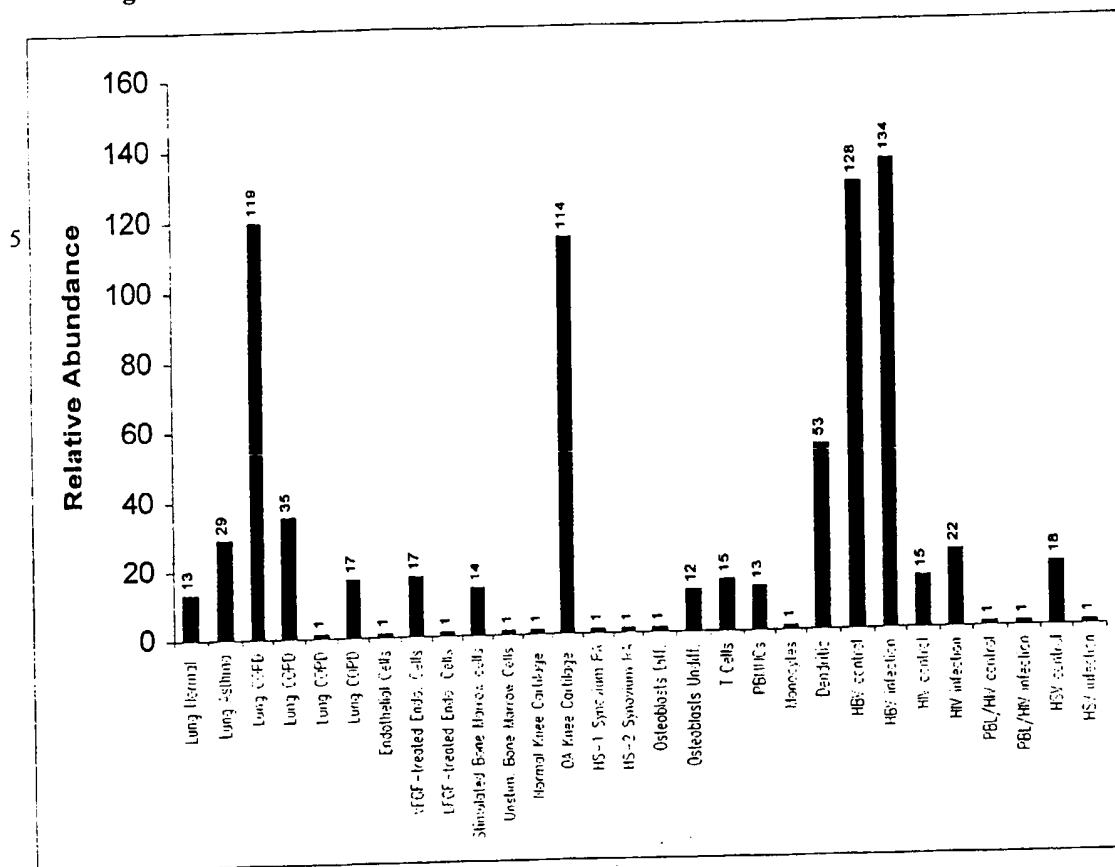


Figure 3: Relative Expression of AXOR87 in Normal and Stimulated Tissues



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1988, 336:783-787), G-proteins themselves, effector proteins, e.g., phospholipase C, adenylyl cyclase, and phosphodiesterase, and actuator proteins, e.g., protein kinase A and protein kinase C (Simon, M.I., et al., Science, 1991, 252:802-8).

For example, in one form of signal transduction, the effect of hormone binding is
5 activation of the enzyme, adenylyl cyclase, inside the cell. Enzyme activation by
hormones is dependent on the presence of the nucleotide, GTP. GTP also influences
hormone binding. A G-protein connects the hormone receptor to adenylyl cyclase. G-
protein was shown to exchange GTP for bound GDP when activated by a hormone receptor.
The GTP-carrying form then binds to activated adenylyl cyclase. Hydrolysis of GTP to
10 GDP, catalyzed by the G-protein itself, returns the G-protein to its basal, inactive form.
Thus, the G-protein serves a dual role, as an intermediate that relays the signal from
receptor to effector, and as a clock that controls the duration of the signal.

The membrane protein gene superfamily of G-protein coupled receptors has been
characterized as having seven putative transmembrane domains. The domains are believed
15 to represent transmembrane α -helices connected by extracellular or cytoplasmic loops. G-
protein coupled receptors include a wide range of biologically active receptors, such as
hormone, viral, growth factor and neuroreceptors.

G-protein coupled receptors (otherwise known as 7TM receptors) have been
characterized as including these seven conserved hydrophobic stretches of about 20 to 30
20 amino acids, connecting at least eight divergent hydrophilic loops. The G-protein family of
coupled receptors includes dopamine receptors which bind to neuroleptic drugs used for
treating psychotic and neurological disorders. Other examples of members of this family
include, but are not limited to, calcitonin, adrenergic, endothelin, cAMP, adenosine,
muscarinic, acetylcholine, serotonin, histamine, thrombin, kinin, follicle stimulating
25 hormone, opsins, endothelial differentiation gene-1, rhodopsins, odorant, and
cytomegalovirus receptors.

Most G-protein coupled receptors have single conserved cysteine residues in each
of the first two extracellular loops which form disulfide bonds that are believed to stabilize
functional protein structure. The 7 transmembrane regions are designated as TM1, TM2,

Phosphorylation and lipidation (palmitoylation or farnesylation) of cysteine residues can influence signal transduction of some G-protein coupled receptors. Most G-protein coupled receptors contain potential phosphorylation sites within the third cytoplasmic loop and/or the carboxy terminus. For several G-protein coupled receptors, such as the β -adrenoreceptor, phosphorylation by protein kinase A and/or specific receptor kinases mediates receptor desensitization.

For some receptors, the ligand binding sites of G-protein coupled receptors are believed to comprise hydrophilic sockets formed by several G-protein coupled receptor transmembrane domains, said sockets being surrounded by hydrophobic residues of the G-protein coupled receptors. The hydrophilic side of each G-protein coupled receptor transmembrane helix is postulated to face inward and form a polar ligand binding site. TM3 has been implicated in several G-protein coupled receptors as having a ligand binding site, such as the TM3 aspartate residue. TM5 serines, a TM6 asparagine and TM6 or TM7 phenylalanines or tyrosines are also implicated in ligand binding.

G-protein coupled receptors can be intracellularly coupled by heterotrimeric G-proteins to various intracellular enzymes, ion channels and transporters (see, Johnson et al., Endoc. Rev., 1989, 10:317-331). Different G-protein α -subunits preferentially stimulate particular effectors to modulate various biological functions in a cell. Phosphorylation of cytoplasmic residues of G-protein coupled receptors has been identified as an important mechanism for the regulation of G-protein coupling of some G-protein coupled receptors. G-protein coupled receptors are found in numerous sites within a mammalian host.

The purinergic family of receptors comprise 5 known human receptors and a large number of related orphan receptors. Tissue distribution analysis for many of these receptors indicates a role in respiratory and immune diseases. For example, P2Y2 controls nucleotide-regulated mucus secretion from goblet and sub-mucosal glands in the lung and is thought to be a therapeutic target for bronchitis. The P2Y1 receptor has potential roles in the brain, lung and immune system based on immunohistochemistry data. Many of these receptors are expressed in human immune cells. For example, P2Y10 is highly expressed in dendritic cells and P2Y6 in T and B cells. HM74 is expressed in immune, smooth

urinary bladder, myometrium, breast adipose, omentum). In the immune system, expression of HM74 is seen in neutrophils and monocytes but not T cells or dendritic cells.

Over the past 15 years, nearly 350 therapeutic agents targeting 7 transmembrane (7 TM) receptors have been successfully introduced onto the market.

5 Summary of the Invention

A novel purinergic-like receptor, referred to herein as AXOR87 (also known as HIPHUM 0000139), is now provided. The present invention relates to AXOR87, in particular AXOR87 polypeptides and AXOR87 polynucleotides, recombinant materials and methods for their production. Such polypeptides and polynucleotides are of interest in
10 relation to methods of treatment of certain diseases related particularly to immunity, autoimmunity, inflammation, immunodeficiency, COPD, and viral infections, and including, but not limited to: infections such as bacterial, fungal, and protozoan infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; diabetes; diabetic retinopathy; obesity; anorexia; bulimia; asthma; rheumatoid arthritis; osteoarthritis;
15 inflammation; inflammatory pain; dermatitis; psoriasis; rhinitis; anaphylaxis; septic shock; Grave's disease; Parkinson's disease; Crohn's disease; Goodpasture's syndrome; acute heart failure; hypotension; hypertension; urinary retention; osteoporosis; angina pectoris; myasthenia gravis; ulcerative colitis; inflammatory bowel syndrome; urinary incontinence; benign prostatic hypertrophy; acute prostatitis; systemic lupus erythematosus; gastritis;
20 diverticulitis; myocardial infarction; ischemic heart disease; peripheral vascular disease; hemangiomas; arteriosclerosis; stroke; angiogenesis; neovascular glaucoma; ulcers; allergies; benign prostatic hypertrophy; migraine; vomiting; cancer metastasis; establishment of tumors; endometriosis; psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, depression, delirium, dementia, and severe mental
25 retardation; and dyskinesias, such as Huntington's disease or Gilles de la Tourette's syndrome hereinafter referred to as "diseases of the invention." In a further aspect, the invention relates to methods for identifying agonists and antagonists (e.g., inhibitors) using the materials provided by the invention, and treating conditions associated with AXOR87 imbalance with the identified compounds. In a still further aspect, the invention relates to
30 diagnostic assays for detecting diseases associated with inappropriate AXOR87 activity or

thymus. The receptor is also expressed in myometrium, ovary, lung, trachea, tonsil, skin, omentum, prostate, rectum and thyroid (Figure 1). The receptor was most highly expressed in thymus tissue which may be due to expression in immune cells, such as T cells, or thymic epithelium. Thymic epithelium plays a role in antigen presentation in T cell development.

5 AXOR87 was most highly expressed in dendritic cells among the immune cells tested (Figure 3). These are antigen presenting cells, as are Langerhans cells which are present in skin where the receptor was also expressed. Thyroid epithelium can also play a role in antigen presentation under certain conditions. AXOR87 mRNA expression was upregulated in all colon tumors versus normal colon. The receptor was down-regulated in

10 all lung tumors versus normal tissue. Differential regulation was seen in breast tumors (Figure 2). AXOR87 mRNA expression was up-regulated in osteoarthritic knee and down-regulated in differentiated osteoblasts. AXOR87 was expressed in dendritic and T cells and expression was up-regulated in growth factor-stimulated bone marrow cells. AXOR87 expression was upregulated in vascular endothelial growth factor-treated cells. (Figure 3).

15 The novel purinergic-like receptor is a screening target for the identification and development of novel pharmaceutical agents, including modulators of purinergic receptor activity.

Brief Description of the Figures

Figure 1 shows the relative expression levels of AXOR87 in human tissues.

20 Figure 2 shows the relative expression of AXOR87 in normal and tumor tissue from the colon, lung and breast.

Figure 3 shows the relative expression of AXOR87 in a variety of normal and stimulated tissues and cells.

Description of the Invention

25 In a first aspect, the present invention relates to AXOR87 polypeptides. Such polypeptides include:

(a) an isolated polypeptide encoded by a polynucleotide comprising the sequence

(b) an isolated polypeptide comprising a polypeptide sequence having at least 95%, 96%, 97%, 98%, or 99% identity to the polypeptide sequence of SEQ ID NO:2;

(c) an isolated polypeptide comprising the polypeptide sequence of SEQ ID NO:2;

(d) an isolated polypeptide having at least 95%, 96%, 97%, 98%, or 99% identity
5 to the polypeptide sequence of SEQ ID NO:2;

(e) the polypeptide sequence of SEQ ID NO:2;

(f) an isolated polypeptide having or comprising a polypeptide sequence that has an Identity Index of 0.95, 0.96, 0.97, 0.98, or 0.99 compared to the polypeptide sequence of SEQ ID NO:2; and

10 (g) fragments and variants of such polypeptides in (a) to (f).

Polypeptides of the present invention are believed to be members of the G-protein Coupled family of polypeptides. They are therefore of interest because 7TM Receptors, more than any other gene family, are the targets of pharmaceutical intervention. The biological properties of the AXOR87 are hereinafter referred to as "biological activity of
15 AXOR87" or "AXOR87 activity". Preferably, a polypeptide of the present invention exhibits at least one biological activity of AXOR87.

Polypeptides of the present invention also includes variants of the aforementioned polypeptides, including all allelic forms and splice variants. Such polypeptides vary from the reference polypeptide by insertions, deletions, and substitutions that may be
20 conservative or non-conservative, or any combination thereof. Particularly preferred variants are those in which several, for instance from 50 to 30, from 30 to 20, from 20 to 10, from 10 to 5, from 5 to 3, from 3 to 2, from 2 to 1 or 1 amino acids are inserted, substituted, or deleted, in any combination. The term "variant" refers to a polypeptide which has a same essential character or basic biological functionality as AXOR87. The essential character of
25 AXOR87 can be defined as follows: AXOR87 is a purinergic-like receptor. Preferably a variant polypeptide is one which binds to the same ligand as AXOR87. Preferably the polypeptide shows immunomodulatory activity or effects on endothelial cell function or angiogenesis. A polypeptide having a same essential character as AXOR87 may be

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regulate immune function, possibly through a role in antigen presentation to and activation of T lymphocytes. A suitable variant may play a role in endothelial cell function and angiogenesis, or may control smooth muscle contraction in tissues such as urinary bladder. A variant of AXOR87 may have a similar activity to HM74 or may act as a co-receptor for HM74. A variant receptor may be identified by looking for ligand binding. Possible ligands include ATP, ADP, and AMP and derivatives thereof such as 2 methylthio-ATP, 2 methylthio-ADP, homo-ATP, ATP γ S, ATP α S, ATP β F, UTP, UDP, $\alpha\beta$ -methyl ATP, Ap2A, Ap3A, Ap4A, Ap6A, CTP, CDP, ITP, IDP, GTP, GDP, TTP, TDP, ADP-glucose, CDP-glucose, TDP-glucose, GDP-glucose, UDP-glucose, UDP-galacturonic acid, UDP-glucuronic acid, UDP-N acetylglucosamine, UDP-mannose, UDP-xylose, ADP-ribose, and/or ATP-ribose.. A full length variant polypeptide is preferably one which includes a seven transmembrane region. Preferably, a full length variant polypeptide may couple to G-protein to mediate intracellular responses.

In another aspect of the invention, a variant is one which does not show the same activity as AXOR87 but is one which inhibits a basic function of AXOR87. For example, a variant polypeptide is one which inhibits the immunomodulatory activity or effects on endothelial cell function or angiogenesis of AXOR87, for example by binding to AXOR87 ligand to prevent activity mediated by ligand binding to AXOR87.

Preferred fragments of polypeptides of the present invention include an isolated polypeptide comprising an amino acid sequence having at least 30, 50 or 100 contiguous amino acids from the amino acid sequence of SEQ ID NO: 2, or an isolated polypeptide comprising an amino acid sequence having at least 30, 50 or 100 contiguous amino acids truncated or deleted from the amino acid sequence of SEQ ID NO: 2. Preferred fragments are biologically active fragments that mediate the biological activity of AXOR87, including those with a similar activity or an improved activity, or with a decreased undesirable activity. Also preferred are those fragments that are antigenic or immunogenic in an animal, especially in a human.

The invention also includes a polypeptide consisting of or comprising a polypeptide of the formula:

wherein each occurrence of R_1 and R_2 is independently any amino acid residue or modified amino acid residue, m is zero or is an integer between 1 and 1000, n is zero or is an integer between 1 and 1000, and SEQ ID NO:2 is an amino acid sequence of the invention. In the formula above, SEQ ID NO:2 is oriented so that its amino terminus is the amino acid residue at the left, covalently bound to R_1 , and its carboxy terminus is the amino acid residue at the right, covalently bound to R_2 . Any stretch of amino acid residues denoted by either R_1 or R_2 , wherein m and/or n is greater than 1, may be either a heteropolymer or a homopolymer, preferably a heteropolymer. Other suitable embodiments of the invention are those wherein m is an integer between 1 and 50, 1 and 100, or 1 and 500, and n is an integer between 1 and 50, 1 and 100, or 1 and 500.

It will be appreciated by those skilled in the art, that in the above identified structure, R_1 or R_2 or both may represent sequences such as a leader or secretory sequence, a pre-, pro- or prepro- protein sequence or the like as further described below.

Fragments of the polypeptides of the invention may be employed for producing the corresponding full-length polypeptide by peptide synthesis; therefore, these variants may be employed as intermediates for producing the full-length polypeptides of the invention. The polypeptides of the present invention may be in the form of the "mature" protein or may be a part of a larger protein such as a precursor or a fusion protein. It is often advantageous to include an additional amino acid sequence that contains secretory or leader sequences, pro-sequences, sequences that aid in purification, for instance multiple histidine residues, or an additional sequence for stability during recombinant production.

Polypeptides of the present invention can be prepared in any suitable manner, for instance by isolation from naturally occurring sources, from genetically engineered host cells comprising expression systems (*vide infra*) or by chemical synthesis, using for instance automated peptide synthesizers, or a combination of such methods. Means for preparing such polypeptides are well understood in the art.

In a further aspect, the present invention relates to AXOR87 polynucleotides. Such polynucleotides include

(a) an isolated polynucleotide comprising a polynucleotide sequence having at least 95%, 96%, 97%, 98%, or 99% identity to the polynucleotide sequence of SEQ ID NO:1;

(b) an isolated polynucleotide comprising the polynucleotide of SEQ ID NO:1;

5 (c) an isolated polynucleotide having at least 95%, 96%, 97%, 98%, or 99% identity to the polynucleotide of SEQ ID NO:1;

(d) the isolated polynucleotide of SEQ ID NO:1;

(e) an isolated polynucleotide comprising a polynucleotide sequence encoding a polypeptide sequence having at least 95%, 96%, 97%, 98%, or 99% identity to the
10 polypeptide sequence of SEQ ID NO:2;

(f) an isolated polynucleotide comprising a polynucleotide sequence encoding the polypeptide of SEQ ID NO:2;

(g) an isolated polynucleotide having a polynucleotide sequence encoding a polypeptide sequence having at least 95%, 96%, 97%, 98%, or 99% identity to the
15 polypeptide sequence of SEQ ID NO:2;

(h) an isolated polynucleotide encoding the polypeptide of SEQ ID NO:2;

(i) an isolated polynucleotide having or comprising a polynucleotide sequence that has an Identity Index of 0.95, 0.96, 0.97, 0.98, or 0.99 compared to the polynucleotide sequence of SEQ ID NO:1;

20 (j) an isolated polynucleotide having or comprising a polynucleotide sequence encoding a polypeptide sequence that has an Identity Index of 0.95, 0.96, 0.97, 0.98, or 0.99 compared to the polypeptide sequence of SEQ ID NO:2; and

polynucleotides that are fragments and variants of the above mentioned polynucleotides or that are complementary to above mentioned polynucleotides, over the entire length
25 thereof.

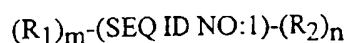
preferred fragments of the above mentioned polynucleotides are those polynucleotides comprising a nucleotide sequence having at least 10, 20, 50, or 100

contiguous nucleotides from the sequence of SEQ ID NO: 1, or an isolated polynucleotide comprising an sequence having at least 30, 50 or 100 contiguous nucleotides truncated or deleted from the sequence of SEQ ID NO: 1.

Preferred variants of polynucleotides of the present invention include splice variants, allelic variants, and polymorphisms, including polynucleotides having one or more single nucleotide polymorphisms (SNPs).

Polynucleotides of the present invention also include polynucleotides encoding polypeptide variants that comprise the amino acid sequence of SEQ ID NO:2 and in which several, for instance from 50 to 30, from 30 to 20, from 20 to 10, from 10 to 5, from 5 to 3, from 3 to 2, from 2 to 1 or 1 amino acid residues are substituted, deleted or added, in any combination.

The invention also includes a polynucleotide consisting of or comprising a polynucleotide of the formula:



wherein, each occurrence of R_1 and R_2 is independently any nucleic acid residue or modified nucleic acid residue, m is zero or an integer between 1 and 3000, n is zero or an integer between 1 and 3000, and SEQ ID NO:1 is a nucleotide sequence of the invention. In the polynucleotide formula above, SEQ ID NO:1 is oriented so that its 5' end nucleic acid residue is at the left, bound to R_1 , and its 3' end nucleic acid residue is at the right, bound to R_2 . Any stretch of nucleic acid residues denoted by R_1 or R_2 , wherein m or n or both are greater than 1, may be either a heteropolymer or a homopolymer, preferably a heteropolymer. Where R_1 and R_2 are joined together by a covalent bond, the polynucleotide of the above formula is a closed, circular polynucleotide, that can be a double-stranded polynucleotide wherein the formula shows a first strand to which the second strand is complementary. In another embodiment m or n or both are an integer between 1 and 1000. Other embodiments of the invention include those wherein m is an integer between 1 and 50, 1 and 100 or 1 and 500, and n is an integer between 1 and 50, 1 and 100, or 1 and 500.

In a further aspect, the present invention provides polynucleotides that are RNA transcripts of the DNA sequences of the present invention. Accordingly, there is provided an RNA polynucleotide that:

(a) comprises an RNA transcript of the DNA sequence encoding the polypeptide
5 of SEQ ID NO:2;

(b) is the RNA transcript of the DNA sequence encoding the polypeptide of SEQ ID NO:2;

(c) comprises an RNA transcript of the DNA sequence of SEQ ID NO:1; or

(d) is the RNA transcript of the DNA sequence of SEQ ID NO:1;

10 and RNA polynucleotides that are complementary thereto.

The polynucleotide sequence of SEQ ID NO:1 shows homology with Human HM74 receptor [H. Nomura, et al., Int. Immunol. 5(10), 1239-1249 (1993)]. The polynucleotide sequence of SEQ ID NO:1 is a cDNA sequence that encodes the polypeptide of SEQ ID NO:2. The polynucleotide sequence encoding the polypeptide of SEQ ID
15 NO:2 may be identical to the polypeptide encoding sequence of SEQ ID NO:1 or it may be a sequence other than SEQ ID NO:1, which, as a result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:2. The polypeptide of SEQ ID NO:2 is related to other proteins of the G-protein Coupled family, having homology and/or structural similarity with Human HM74 receptor [H. Nomura, et
20 al., Int. Immunol., 5(10), 1239-1249 (1993)].

Preferred polypeptides and polynucleotides of the present invention are expected to have, *inter alia*, similar biological functions/properties to their homologous polypeptides and polynucleotides. Furthermore, preferred polypeptides and polynucleotides of the present invention have at least one AXOR87 activity.

25 Polynucleotides of the present invention may be obtained using standard cloning and screening techniques from a cDNA library derived from mRNA in cells of human brain

Goldstein, Sambrook, et al., Molecular Cloning: A Laboratory Manual, 2nd Ed
© 1989 Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY 11724. Polynucleotide

of the invention can also be obtained from natural sources such as genomic DNA libraries or can be synthesized using well known and commercially available techniques.

When polynucleotides of the present invention are used for the recombinant production of polypeptides of the present invention, the polynucleotide may include the coding sequence for the mature polypeptide, by itself, or the coding sequence for the mature polypeptide in reading frame with other coding sequences, such as those encoding a leader or secretory sequence, a pre-, or pro- or prepro- protein sequence, or other fusion peptide portions. For example, a marker sequence that facilitates purification of the fused polypeptide can be encoded. In certain preferred embodiments of this aspect of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz *et al.*, Proc Natl Acad Sci USA (1989) 86:821-824, or is an HA tag. The polynucleotide may also contain non-coding 5' and 3' sequences, such as transcribed, non-translated sequences, splicing and polyadenylation signals, ribosome binding sites and sequences that stabilize mRNA.

Polynucleotides that are identical, or have sufficient identity to a polynucleotide sequence of SEQ ID NO:1, may be used as hybridization probes for cDNA and genomic DNA or as primers for a nucleic acid amplification reaction (for instance, PCR). Such probes and primers may be used to isolate full-length cDNAs and genomic clones encoding polypeptides of the present invention and to isolate cDNA and genomic clones of other genes (including genes encoding paralogs from human sources and orthologs and paralogs from species other than human) that have a high sequence similarity to SEQ ID NO:1, typically at least 95% identity. Preferred probes and primers will generally comprise at least 15 nucleotides, preferably, at least 30 nucleotides and may have at least 50, if not at least 100 nucleotides. Particularly preferred probes will have between 30 and 50 nucleotides. Particularly preferred primers will have between 20 and 25 nucleotides.

A polynucleotide encoding a polypeptide of the present invention, including homologs from species other than human, may be obtained by a process comprising the steps of screening a library under stringent hybridization conditions with a labeled probe having the sequence of SEQ ID NO: 1 or a fragment thereof, preferably of at least 15 nucleotides; and isolating full-length cDNA and genomic clones containing said

artisan. Preferred stringent hybridization conditions include overnight incubation at 42°C in a solution comprising: 50% formamide, 5xSSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10 % dextran sulfate, and 20 microgram/ml denatured, sheared salmon sperm DNA; followed by washing the filters in
5 0.1x SSC at about 65°C. Thus the present invention also includes isolated polynucleotides, preferably with a nucleotide sequence of at least 100, obtained by screening a library under stringent hybridization conditions with a labeled probe having the sequence of SEQ ID NO:1 or a fragment thereof, preferably of at least 15 nucleotides.

The skilled artisan will appreciate that, in many cases, an isolated cDNA
10 sequence will be incomplete, in that the region coding for the polypeptide does not extend all the way through to the 5' terminus. This is a consequence of reverse transcriptase, an enzyme with inherently low "processivity" (a measure of the ability of the enzyme to remain attached to the template during the polymerisation reaction), failing to complete a DNA copy of the mRNA template during first strand cDNA synthesis.

15 There are several methods available and well known to those skilled in the art to obtain full-length cDNAs, or extend short cDNAs, for example those based on the method of Rapid Amplification of cDNA ends (RACE) (see, for example, Frohman et al., Proc Nat Acad Sci USA 85, 8998-9002, 1988). Recent modifications of the technique, exemplified by the Marathon (trade mark) technology (Clontech Laboratories Inc.) for
20 example, have significantly simplified the search for longer cDNAs. In the Marathon (trade mark) technology, cDNAs have been prepared from mRNA extracted from a chosen tissue and an 'adaptor' sequence ligated onto each end. Nucleic acid amplification (PCR) is then carried out to amplify the "missing" 5' end of the cDNA using a combination of gene specific and adaptor specific oligonucleotide primers. The PCR
25 reaction is then repeated using 'nested' primers, that is, primers designed to anneal within the amplified product (typically an adapter specific primer that anneals further 3' in the adaptor sequence and a gene specific primer that anneals further 5' in the known gene sequence). The products of this reaction can then be analyzed by DNA sequencing and a full-length cDNA constructed either by joining the product directly to the existing cDNA
30 to give a complete sequence, or carrying out a separate full-length PCR using the new

Recombinant polypeptides of the present invention may be prepared by processes well known in the art from genetically engineered host cells comprising expression systems. Accordingly, in a further aspect, the present invention relates to expression systems comprising a polynucleotide or polynucleotides of the present invention, to host cells which are genetically engineered with such expression systems and to the production of polypeptides of the invention by recombinant techniques. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention.

For recombinant production, host cells can be genetically engineered to incorporate expression systems or portions thereof for polynucleotides of the present invention. Polynucleotides may be introduced into host cells by methods described in many standard laboratory manuals, such as Davis et al, Basic Methods in Molecular Biology (1986) and Sambrook *et al. (ibid)*. Preferred methods of introducing polynucleotides into host cells include, for instance, calcium phosphate transfection, DEAE-dextran mediated transfection, transfection, micro-injection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction or infection.

Representative examples of appropriate hosts include bacterial cells, such as *Streptococci*, *Staphylococci*, *E. coli*, *Streptomyces* and *Bacillus subtilis* cells; fungal cells, such as yeast cells and *Aspergillus* cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, HEK 293 and Bowes melanoma cells; and plant cells.

A great variety of expression systems can be used, for instance, chromosomal, episomal and virus-derived systems, *e.g.*, vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids. The expression systems may contain control regions that regulate as well as engender expression. Generally, any system or vector that is able to maintain, propagate or express a polynucleotide to produce a

inserted into an expression system by any of a variety of well-known and routine techniques, such as, for example, those set forth in Sambrook *et al.*, (*ibid*). Appropriate secretion signals may be incorporated into the desired polypeptide to allow secretion of the translated protein into the lumen of the endoplasmic reticulum, the periplasmic space or the extracellular environment. These signals may be endogenous to the polypeptide or they may be heterologous signals.

If a polypeptide of the present invention is to be expressed for use in screening assays, it is generally preferred that the polypeptide be produced at the surface of the cell. In this event, the cells may be harvested prior to use in the screening assay. If the polypeptide is secreted into the medium, the medium can be recovered in order to recover and purify the polypeptide. If produced intracellularly, the cells must first be lysed before the polypeptide is recovered.

Polypeptides of the present invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography is employed for purification. Well known techniques for refolding proteins may be employed to regenerate active conformation when the polypeptide is denatured during intracellular synthesis, isolation and/or purification.

Polynucleotides of the present invention may be used as diagnostic reagents, through detecting mutations in the associated gene. Detection of a mutated form of the gene characterized by the polynucleotide of SEQ ID NO:1 in the cDNA or genomic sequence and which is associated with a dysfunction will provide a diagnostic tool that can add to, or define, a diagnosis of a disease, or susceptibility to a disease, which results from under-expression, over-expression or altered spatial or temporal expression of the gene. Individuals carrying mutations in the gene may be detected at the DNA level by a variety of techniques well known in the art.

Nucleic acids for diagnosis may be obtained from a subject's cells, such as from directly for detection or it may be amplified enzymatically by using PCR, preferably by

PCR, or other amplification techniques prior to analysis. RNA or cDNA may also be used in similar fashion. Deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to labeled AXOR87 nucleotide sequences. Perfectly
5 matched sequences can be distinguished from mismatched duplexes by RNase digestion or by differences in melting temperatures. DNA sequence difference may also be detected by alterations in the electrophoretic mobility of DNA fragments in gels, with or without denaturing agents, or by direct DNA sequencing (see, for instance, Myers *et al.*, Science (1985) 230:1242). Sequence changes at specific locations may also be revealed by nuclease
10 protection assays, such as RNase and S1 protection or the chemical cleavage method (see Cotton *et al.*, Proc Natl Acad Sci USA (1985) 85: 4397-4401).

An array of oligonucleotide probes comprising AXOR87 polynucleotide sequence or fragments thereof can be constructed to conduct efficient screening of *e.g.*, genetic mutations. Such arrays are preferably high density arrays or grids. Array technology
15 methods are well known and have general applicability and can be used to address a variety of questions in molecular genetics including gene expression, genetic linkage, and genetic variability, see, for example, M. Chee *et al.*, Science, 274, 610-613 (1996) and other references cited therein.

Detection of abnormally decreased or increased levels of polypeptide or mRNA
20 expression may also be used for diagnosing or determining susceptibility of a subject to a disease of the invention. Decreased or increased expression can be measured at the RNA level using any of the methods well known in the art for the quantitation of polynucleotides, such as, for example, nucleic acid amplification, for instance PCR, RT-PCR, RNase protection, Northern blotting and other hybridization methods. Assay
25 techniques that can be used to determine levels of a protein, such as a polypeptide of the present invention, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radio-immunoassays, competitive-binding assays, Western Blot analysis and ELISA assays.

Thus in another aspect, the present invention relates to a diagnostic kit
30 comprising:

(a) a polynucleotide of the present invention, preferably the nucleotide sequence of SEQ ID NO: 1, or a fragment or an RNA transcript thereof;

(b) a nucleotide sequence complementary to that of (a);

(c) a polypeptide of the present invention, preferably the polypeptide of SEQ ID NO:2 or a fragment thereof; or

(d) an antibody to a polypeptide of the present invention, preferably to the polypeptide of SEQ ID NO:2.

It will be appreciated that in any such kit, (a), (b), (c) or (d) may comprise a substantial component. Such a kit will be of use in diagnosing a disease or susceptibility to a disease, particularly diseases of the invention, amongst others.

The polynucleotide sequences of the present invention are valuable for chromosome localisation studies. The sequence is specifically targeted to, and can hybridize with, a particular location on an individual human chromosome. The mapping of relevant sequences to chromosomes according to the present invention is an important first step in correlating those sequences with gene associated disease. Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found in, for example, V. McKusick, Mendelian Inheritance in Man (available on-line through Johns Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (co-inheritance of physically adjacent genes). Precise human chromosomal localisations for a genomic sequence (gene fragment etc.) can be determined using Radiation Hybrid (RH) Mapping (Walter, M. Spillett, D., Thomas, P., Weissenbach, J., and Goodfellow, P., (1994) A method for constructing radiation hybrid maps of whole genomes, Nature Genetics 7, 22-28). A number of RH panels are available from Research Genetics (Huntsville, AL, USA) e.g. the GeneBridge4 RH panel (Hum Mol Genet 1996 Mar;5(3):339-46 A radiation hybrid map of the human genome. Gyapay G, Schmitt K, Fizames C, Jones H, Vega-Czarny N, Spillett D, Muselet D, Prud'Homme JF, Dib C,

designed from the gene of interest on RH DNAs. Each of these DNAs contains random human genomic fragments maintained in a hamster background (human / hamster hybrid cell lines). These PCRs result in 93 scores indicating the presence or absence of the PCR product of the gene of interest. These scores are compared with scores created using
5 PCR products from genomic sequences of known location. This comparison is conducted at <http://www.genome.wi.mit.edu/>. The gene of the present invention maps to human chromosome 12q.

The polynucleotide sequences of the present invention are also valuable tools for tissue expression studies. Such studies allow the determination of expression patterns of
10 polynucleotides of the present invention which may give an indication as to the expression patterns of the encoded polypeptides in tissues, by detecting the mRNAs that encode them. The techniques used are well known in the art and include in situ hybridization techniques to clones arrayed on a grid, such as cDNA microarray hybridization (Schena *et al*, Science, 270, 467-470, 1995 and Shalon *et al*, Genome Res, 6, 639-645, 1996) and nucleotide
15 amplification techniques such as PCR. A preferred method uses the TAQMAN (Trade mark) technology available from Perkin Elmer. Results from these studies can provide an indication of the normal function of the polypeptide in the organism. In addition, comparative studies of the normal expression pattern of mRNAs with that of mRNAs encoded by an alternative form of the same gene (for example, one having an alteration in
20 polypeptide coding potential or a regulatory mutation) can provide valuable insights into the role of the polypeptides of the present invention, or that of inappropriate expression thereof in disease. Such inappropriate expression may be of a temporal, spatial or simply quantitative nature. The polypeptides of the present invention are expressed in brain and testis.

25 A further aspect of the present invention relates to antibodies. The polypeptides of the invention or their fragments, or cells expressing them, can be used as immunogens to produce antibodies that are immunospecific for polypeptides of the present invention. The term "immunospecific" means that the antibodies have substantially greater affinity for the polypeptides of the invention than their affinity for other related polypeptides in the prior
30 art.

Antibodies generated against polypeptides of the present invention may be obtained by administering the polypeptides or epitope-bearing fragments, or cells to an animal, preferably a non-human animal, using routine protocols. For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler, G. and Milstein, C., *Nature* (1975) 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor *et al.*, *Immunology Today* (1983) 4:72) and the EBV-hybridoma technique (Cole *et al.*, *Monoclonal Antibodies and Cancer Therapy*, 77-96, Alan R. Liss, Inc., 1985).

Techniques for the production of single chain antibodies, such as those described in U.S. Patent No. 4,946,778, can also be adapted to produce single chain antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms, including other mammals, may be used to express humanized antibodies.

The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptide or to purify the polypeptides by affinity chromatography. Antibodies against polypeptides of the present invention may also be employed to treat diseases of the invention, amongst others.

Polypeptides and polynucleotides of the present invention may also be used as vaccines. Accordingly, in a further aspect, the present invention relates to a method for inducing an immunological response in a mammal that comprises inoculating the mammal with a polypeptide of the present invention, adequate to produce antibody and/or T cell immune response, including, for example, cytokine-producing T cells or cytotoxic T cells, to protect said animal from disease, whether that disease is already established within the individual or not. An immunological response in a mammal may also be induced by a method comprises delivering a polypeptide of the present invention *via* a vector directing expression of the polynucleotide and coding for the polypeptide *in vivo* in order to induce such an immunological response to produce antibody to protect said animal from diseases of the invention. One way of administering the vector is by accelerating it into the desired cells as a coating on particles or otherwise. Such nucleic acid vector may comprise DNA, RNA, a modified nucleic acid, or a DNA/RNA hybrid

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vaccine formulation (composition). The formulation may further comprise a suitable carrier. Since a polypeptide may be broken down in the stomach, it is preferably administered parenterally (for instance, subcutaneous, intra-muscular, intravenous, or intra-dermal injection). Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions that may contain anti-oxidants, buffers, bacteriostats and solutes that render the formulation isotonic with the blood of the recipient; and aqueous and non-aqueous sterile suspensions that may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use. The vaccine formulation may also include adjuvant systems for enhancing the immunogenicity of the formulation, such as oil-in water systems and other systems known in the art. The dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation.

Polypeptides of the present invention have one or more biological functions that are of relevance in one or more disease states, in particular the diseases of the invention hereinbefore mentioned. It is therefore useful to identify compounds that stimulate or inhibit the function or level of the polypeptide. Accordingly, in a further aspect, the present invention provides for a method of screening compounds to identify those that stimulate or inhibit the function or level of the polypeptide. Such methods identify agonists or antagonists that may be employed for therapeutic and prophylactic purposes for such diseases of the invention as hereinbefore mentioned. Compounds may be identified from a variety of sources, for example, cells, cell-free preparations, chemical libraries, collections of chemical compounds, and natural product mixtures. Such agonists or antagonists so-identified may be natural or modified substrates, ligands, receptors, enzymes, etc., as the case may be, of the polypeptide; a structural or functional mimetic thereof (see Coligan *et al.*, Current Protocols in Immunology 1(2):Chapter 5 (1991)) or a small molecule. Such small molecules preferably have a molecular weight below 2,000 daltons, more preferably between 300 and 1,000 daltons, and most preferably between 400 and 700 daltons. It is preferred that these small molecules are organic molecules.

The screening method may simply measure the binding of a candidate compound

to the polypeptide or to a derivative of the polypeptide.

thereof, by means of a label directly or indirectly associated with the candidate compound. Alternatively, the screening method may involve measuring or detecting (qualitatively or quantitatively) the competitive binding of a candidate compound to the polypeptide against a labeled competitor (e.g. agonist or antagonist). Further, these
5 screening methods may test whether the candidate compound results in a signal generated by activation or inhibition of the polypeptide, using detection systems appropriate to the cells bearing the polypeptide. Inhibitors of activation are generally assayed in the presence of a known agonist and the effect on activation by the agonist by the presence of the candidate compound is observed. Further, the screening methods may simply
10 comprise the steps of mixing a candidate compound with a solution containing a polypeptide of the present invention, to form a mixture, measuring a AXOR87 activity in the mixture, and comparing the AXOR87 activity of the mixture to a control mixture which contains no candidate compound.

Polypeptides of the present invention may be employed in conventional low
15 capacity screening methods and also in high-throughput screening (HTS) formats. Such HTS formats include not only the well-established use of 96- and, more recently, 384-well micotiter plates but also emerging methods such as the nanowell method described by Schullek et al, Anal Biochem., 246, 20-29, (1997).

Fusion proteins, such as those made from Fc portion and AXOR87 polypeptide,
20 as hereinbefore described, can also be used for high-throughput screening assays to identify antagonists for the polypeptide of the present invention (see D. Bennett *et al.*, J Mol Recognition, 8:52-58 (1995); and K. Johanson *et al.*, J Biol Chem, 270(16):9459-9471 (1995)).

One screening technique includes the use of cells which express the receptor of
25 this invention (for example, transfected CHO cells) in a system which measures extracellular pH or intracellular calcium changes caused by receptor activation. In this technique, compounds may be contacted with cells expressing the receptor polypeptide of the present invention. A second messenger response, e.g., signal transduction, pH changes, or changes in calcium level, is then measured to determine whether the potential
30 compound activates or inhibits the receptor.

Another method involves screening for receptor inhibitors by determining inhibition or stimulation of receptor-mediated cAMP and/or adenylate cyclase accumulation. Such a method involves transfecting a eukaryotic cell with the receptor of this invention to express the receptor on the cell surface. The cell is then exposed to potential antagonists in the presence of the receptor of this invention. The amount of cAMP accumulation is then measured. If the potential antagonist binds the receptor, and thus inhibits receptor binding, the levels of receptor-mediated cAMP, or adenylate cyclase, activity will be reduced or increased. Another method for detecting agonists or antagonists for the receptor of the present invention is the yeast based technology as described in U.S. Patent No. 5,482,835.

The polynucleotides, polypeptides and antibodies to the polypeptide of the present invention may also be used to configure screening methods for detecting the effect of added compounds on the production of mRNA and polypeptide in cells. For example, an ELISA assay may be constructed for measuring secreted or cell associated levels of polypeptide using monoclonal and polyclonal antibodies by standard methods known in the art. This can be used to discover agents that may inhibit or enhance the production of polypeptide (also called antagonist or agonist, respectively) from suitably manipulated cells or tissues.

A polypeptide of the present invention may be used to identify membrane bound or soluble receptors, if any, through standard receptor binding techniques known in the art. These include, but are not limited to, ligand binding and crosslinking assays in which the polypeptide is labeled with a radioactive isotope (for instance, ^{125}I), chemically modified (for instance, biotinylated), or fused to a peptide sequence suitable for detection or purification, and incubated with a source of the putative receptor (cells, cell membranes, cell supernatants, tissue extracts, bodily fluids). Other methods include biophysical techniques such as surface plasmon resonance and spectroscopy. These screening methods may also be used to identify agonists and antagonists of the polypeptide that compete with the binding of the polypeptide to its receptors, if any. Standard methods for conducting such assays are well understood in the art.

Examples of antagonists of polypeptides of the present invention include antibodies

substrates, receptors, enzymes, etc., as the case may be, of the polypeptide, *e.g.*, a fragment of the ligands, substrates, receptors, enzymes, etc.; or a small molecule that bind to the polypeptide of the present invention but do not elicit a response, so that the activity of the polypeptide is prevented.

5 Typically, AXOR87 receptor activity can be monitored indirectly for example by measuring a G-protein coupled readout. G-protein coupled readout can typically be monitored using an electrophysiological method to determine the activity of G-protein regulated Ca²⁺ or K⁺ channels or by using a fluorescent dye to measure changed in intracellular Ca²⁺ levels. The receptor could be coupled to Gs, Gq, Gi and/or Go. Thus
10 cAMP or GTP γ S levels or activity, calcium mobilization, inositol triphosphate generation and protein kinase C activation may be monitored.

 Following purinergic-like receptor stimulation, cyclic AMP accumulation can be measured for example in forskolin stimulated CHO cells transformed with the AXOR87 receptor either directly, or indirectly by monitoring the expression of cotransfected reporter
15 gene, the expression of which will be controlled by cyclic AMP response elements.

 Xenopus dermal melanophores aggregate or disperse pigment in response to the activation or inhibition of G-protein coupled receptors. This feature can be exploited as an assay for receptor activation or inhibition if a specific G-protein coupled receptor is exogenously expressed.

20 AXOR87 receptor is likely to couple to G-protein with consequent hydrolysis of GTP. Accumulation of a labelled GTP stable analogue can be measured utilising membrane fractions from overexpression of AXOR87 receptor either in *X. laevis* oocytes or cell lines such as HEK293, CHO, COS7, HeLa on exposure to agonist ligand.

 G-protein coupled receptors have been shown to activate MAPK signalling
25 pathways. Cell lines overexpressing the purinergic-like receptor of the invention with MAPK reporter genes may be utilised as assays for receptor activation or inhibition. The purinergic-like receptor of the invention may be heterologously expressed in modified yeast strains containing multiple reporter genes, such as FUS1-HIS3 and FUS1-lacZ, each linked

It will be appreciated that in any such kit, (a), (b), (c) or (d) may comprise a substantial component.

Glossary

The following definitions are provided to facilitate understanding of certain terms
5 used frequently hereinbefore.

"Antibodies" as used herein includes polyclonal and monoclonal antibodies, chimeric, single chain, and humanized antibodies, as well as Fab fragments, including the products of a Fab or other immunoglobulin expression library.

"Isolated" means altered "by the hand of man" from its natural state, *i.e.*, if it occurs
10 in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living organism is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein. Moreover, a polynucleotide or polypeptide that is introduced into an organism by transformation,
15 genetic manipulation or by any other recombinant method is "isolated" even if it is still present in said organism, which organism may be living or non-living.

"Polynucleotide" generally refers to any polyribonucleotide (RNA) or polydeoxribonucleotide (DNA), which may be unmodified or modified RNA or DNA. "Polynucleotides" include, without limitation, single- and double-stranded DNA, DNA
20 that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions comprising RNA or DNA or both RNA
25 and DNA. The term "polynucleotide" also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications may be made to DNA and RNA; thus,

RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides.

"Polypeptide" refers to any polypeptide comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres.

5 "Polypeptide" refers to both short chains, commonly referred to as peptides, oligopeptides or oligomers, and to longer chains, generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids. "Polypeptides" include amino acid sequences modified either by natural processes, such as post-translational processing, or by chemical modification techniques that are well
10 known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications may occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present to the same or varying degrees at several sites in a given
15 polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched and branched cyclic polypeptides may result from post-translation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, biotinylation,
20 covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI
25 anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination (see, for instance, *Proteins - Structure and Molecular Properties*, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York, 1993;
30 Wold, F., *Post-translational Protein Modifications: Perspectives and Prospects*, 1-12, in *Post-translational Covalent Modification of Proteins*, B. C. Johnson, Ed., Academic

cofactors", Meth Enzymol, 182, 626-646, 1990, and Rattan *et al.*, "Protein Synthesis: Post-translational Modifications and Aging", Ann NY Acad Sci, 663, 48-62, 1992).

"Fragment" of a polypeptide sequence refers to a polypeptide sequence that is shorter than the reference sequence but that retains essentially the same biological function or activity as the reference polypeptide. "Fragment" of a polynucleotide sequence refers to a polynucleotide sequence that is shorter than the reference sequence of SEQ ID NO:1.

"Variant" refers to a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide, but retains the essential properties thereof. A typical variant of a polynucleotide differs in nucleotide sequence from the reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from the reference polypeptide. Generally, alterations are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, insertions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. Typical conservative substitutions include Gly, Ala; Val, Ile, Leu; Asp, Glu; Asn, Gln; Ser, Thr; Lys, Arg; and Phe and Tyr. A variant of a polynucleotide or polypeptide may be naturally occurring such as an allele, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis. Also included as variants are polypeptides having one or more post-translational modifications, for instance glycosylation, phosphorylation, methylation, ADP ribosylation and the like. Embodiments include methylation of the N-terminal amino acid, phosphorylations of serines and threonines and modification of C-terminal glycines.

"Polymorphism" refers to a variation in nucleotide sequence (and encoded polypeptide sequence, if relevant) at a given position in the genome within a population.

"Single Nucleotide Polymorphism" (SNP) refers to the occurrence of nucleotide variability at a single nucleotide position in the genome, within a population. An SNP
5 may occur within a gene or within intergenic regions of the genome. SNPs can be assayed using Allele Specific Amplification (ASA). For the process at least 3 primers are required. A common primer is used in reverse complement to the polymorphism being assayed. This common primer can be between 50 and 1500 bps from the polymorphic
10 base. The other two (or more) primers are identical to each other except that the final 3' base wobbles to match one of the two (or more) alleles that make up the polymorphism. Two (or more) PCR reactions are then conducted on sample DNA, each using the common primer and one of the Allele Specific Primers.

"Splice Variant" as used herein refers to cDNA molecules produced from RNA molecules initially transcribed from the same genomic DNA sequence but which have
15 undergone alternative RNA splicing. Alternative RNA splicing occurs when a primary RNA transcript undergoes splicing, generally for the removal of introns, which results in the production of more than one mRNA molecule each of that may encode different amino acid sequences. The term splice variant also refers to the proteins encoded by the above cDNA molecules.

20 "Identity" reflects a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, determined by comparing the sequences. In general, identity refers to an exact nucleotide to nucleotide or amino acid to amino acid correspondence of the two polynucleotide or two polypeptide sequences, respectively, over the length of the sequences being compared.

25 "% Identity" - For sequences where there is not an exact correspondence, a "% identity" may be determined. In general, the two sequences to be compared are aligned to give a maximum correlation between the sequences. This may include inserting "gaps" in either one or both sequences, to enhance the degree of alignment. A % identity may be determined over the whole length of each of the sequences being compared (so-called

length, or over shorter, defined lengths (so-called local alignment), that is more suitable for sequences of unequal length.

"Similarity" is a further, more sophisticated measure of the relationship between two polypeptide sequences. In general, "similarity" means a comparison between the amino acids of two polypeptide chains, on a residue by residue basis, taking into account not only exact correspondences between a between pairs of residues, one from each of the sequences being compared (as for identity) but also, where there is not an exact correspondence, whether, on an evolutionary basis, one residue is a likely substitute for the other. This likelihood has an associated "score" from which the "% similarity" of the two sequences can then be determined.

Methods for comparing the identity and similarity of two or more sequences are well known in the art. Thus for instance, programs available in the Wisconsin Sequence Analysis Package, version 9.1 (Devereux J et al, Nucleic Acids Res, 12, 387-395, 1984, available from Genetics Computer Group, Madison, Wisconsin, USA), for example the programs BESTFIT and GAP, may be used to determine the % identity between two polynucleotides and the % identity and the % similarity between two polypeptide sequences. BESTFIT uses the "local homology" algorithm of Smith and Waterman (J Mol Biol, 147,195-197, 1981, Advances in Applied Mathematics, 2, 482-489, 1981) and finds the best single region of similarity between two sequences. BESTFIT is more suited to comparing two polynucleotide or two polypeptide sequences that are dissimilar in length, the program assuming that the shorter sequence represents a portion of the longer. In comparison, GAP aligns two sequences, finding a "maximum similarity", according to the algorithm of Needleman and Wunsch (J Mol Biol, 48, 443-453, 1970). GAP is more suited to comparing sequences that are approximately the same length and an alignment is expected over the entire length. Preferably, the parameters "Gap Weight" and "Length Weight" used in each program are 50 and 3, for polynucleotide sequences and 12 and 4 for polypeptide sequences, respectively. Preferably, % identities and similarities are determined when the two sequences being compared are optimally aligned.

Other programs for determining identity and/or similarity between sequences are known in the art. For instance the BLAST (Basic Local Alignment Search Tool) program of Altschul et al (J Mol Biol, 215, 403-416, 1990) is well known in the art.

Mol Biol, 215, 403-410, 1990, Altschul S F et al, Nucleic Acids Res., 25:389-3402, 1997, available from the National Center for Biotechnology Information (NCBI), Bethesda, Maryland, USA and accessible through the home page of the NCBI at www.ncbi.nlm.nih.gov) and FASTA (Pearson W R, Methods in Enzymology, 183, 63-99, 1990; Pearson W R and Lipman D J, Proc Nat Acad Sci USA, 85, 2444-2448, 1988, available as part of the Wisconsin Sequence Analysis Package).

Preferably, the BLOSUM62 amino acid substitution matrix (Henikoff S and Henikoff J G, Proc. Nat. Acad Sci. USA, 89, 10915-10919, 1992) is used in polypeptide sequence comparisons including where nucleotide sequences are first translated into amino acid sequences before comparison.

Preferably, the program BESTFIT is used to determine the % identity of a query polynucleotide or a polypeptide sequence with respect to a reference polynucleotide or a polypeptide sequence, the query and the reference sequence being optimally aligned and the parameters of the program set at the default value, as hereinbefore described.

"Identity Index" is a measure of sequence relatedness which may be used to compare a candidate sequence (polynucleotide or polypeptide) and a reference sequence. Thus, for instance, a candidate polynucleotide sequence having, for example, an Identity Index of 0.95 compared to a reference polynucleotide sequence is identical to the reference sequence except that the candidate polynucleotide sequence may include on average up to five differences per each 100 nucleotides of the reference sequence. Such differences are selected from the group consisting of at least one nucleotide deletion, substitution, including transition and transversion, or insertion. These differences may occur at the 5' or 3' terminal positions of the reference polynucleotide sequence or anywhere between these terminal positions, interspersed either individually among the nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence. In other words, to obtain a polynucleotide sequence having an Identity Index of 0.95 compared to a reference polynucleotide sequence, an average of up to 5 in every 100 of the nucleotides of the in the reference sequence may be deleted, substituted or inserted, or any combination thereof, as hereinbefore described. The same applies *mutatis mutandis* for other values of the Identity Index, for instance 0.96, 0.97.

Similarly, for a polypeptide, a candidate polypeptide sequence having, for example, an Identity Index of 0.95 compared to a reference polypeptide sequence is identical to the reference sequence except that the polypeptide sequence may include an average of up to five differences per each 100 amino acids of the reference sequence.

5 Such differences are selected from the group consisting of at least one amino acid deletion, substitution, including conservative and non-conservative substitution, or insertion. These differences may occur at the amino- or carboxy-terminal positions of the reference polypeptide sequence or anywhere between these terminal positions, interspersed either individually among the amino acids in the reference sequence or in

10 one or more contiguous groups within the reference sequence. In other words, to obtain a polypeptide sequence having an Identity Index of 0.95 compared to a reference polypeptide sequence, an average of up to 5 in every 100 of the amino acids in the reference sequence may be deleted, substituted or inserted, or any combination thereof, as hereinbefore described. The same applies *mutatis mutandis* for other values of the

15 Identity Index, for instance 0.96, 0.97, 0.98 and 0.99.

The relationship between the number of nucleotide or amino acid differences and the Identity Index may be expressed in the following equation:

$$n_a \leq x_a - (x_a \bullet I),$$

in which:

20 n_a is the number of nucleotide or amino acid differences,

x_a is the total number of nucleotides or amino acids in SEQ ID NO:1 or SEQ ID NO:2, respectively,

I is the Identity Index,

\bullet is the symbol for the multiplication operator, and

25 in which any non-integer product of x_a and I is rounded down to the nearest integer prior to

subtracting it from x_a

"Homolog" is a generic term used in the art to indicate a polynucleotide or polypeptide sequence possessing a high degree of sequence relatedness to a reference sequence. Such relatedness may be quantified by determining the degree of identity and/or similarity between the two sequences as hereinbefore defined. Falling within this
5 generic term are the terms "ortholog", and "paralog". "Ortholog" refers to a polynucleotide or polypeptide that is the functional equivalent of the polynucleotide or polypeptide in another species. "Paralog" refers to a polynucleotide or polypeptide that within the same species which is functionally similar.

"Fusion protein" refers to a protein encoded by two, often unrelated, fused genes
10 or fragments thereof. In one example, EP-A-0 464 533-A discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, employing an immunoglobulin Fc region as a part of a fusion protein is advantageous for use in therapy and diagnosis resulting in, for example, improved pharmacokinetic properties [see, *e.g.*,
15 EP-A 0232 262]. On the other hand, for some uses it would be desirable to be able to delete the Fc part after the fusion protein has been expressed, detected and purified.

All publications and references, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference in their entirety as if each individual publication or reference were specifically and individually indicated to
20 be incorporated by reference herein as being fully set forth. Any patent application to which this application claims priority is also incorporated by reference herein in its entirety in the manner described above for publications and references.

Examples

Example 1: Mammalian Cell Expression

25 The receptors of the present invention are expressed in either human embryonic kidney 293 (HEK293) cells or adherent dhfr CHO cells. To maximize receptor expression, typically all 5' and 3' untranslated regions (UTRs) are removed from the receptor cDNA prior to insertion into a pCDN or pCDNA3 vector. The cells are transfected with individual receptor cDNAs by lipofectin and selected in the presence of 400 mg/ml G418. After 3

HEK293 or CHO cells transfected with the vector alone serve as negative controls. To isolate cell lines stably expressing the individual receptors, about 24 clones are typically selected and analyzed by Northern blot analysis. Receptor mRNAs are generally detectable in about 50% of the G418-resistant clones analyzed.

5 Example 2 Ligand bank for binding and functional assays.

A bank of over 600 putative receptor ligands has been assembled for screening. The bank comprises: transmitters, hormones and chemokines known to act via a human seven transmembrane (7TM) receptor; naturally occurring compounds which may be putative agonists for a human 7TM receptor, non-mammalian, biologically active peptides
10 for which a mammalian counterpart has not yet been identified; and compounds not found in nature, but which activate 7TM receptors with unknown natural ligands. This bank is used to initially screen the receptor for known ligands, using both functional (i.e., calcium, cAMP, microphysiometer, oocyte electrophysiology, etc, see below) as well as binding assays.

15 Example 3: Ligand Binding Assays

Ligand binding assays provide a direct method for ascertaining receptor pharmacology and are adaptable to a high throughput format. The purified ligand for a receptor is radiolabeled to high specific activity (50-2000 Ci/mmol) for binding studies. A determination is then made that the process of radiolabeling does not diminish the activity of
20 the ligand towards its receptor. Assay conditions for buffers, ions, pH and other modulators such as nucleotides are optimized to establish a workable signal to noise ratio for both membrane and whole cell receptor sources. For these assays, specific receptor binding is defined as total associated radioactivity minus the radioactivity measured in the presence of an excess of unlabeled competing ligand. Where possible, more than one competing ligand
25 is used to define residual nonspecific binding.

Example 4: Functional Assay in *Xenopus* Oocytes

Capped RNA transcripts from linearized plasmid templates encoding the receptor cDNAs of the invention are synthesized in vitro with RNA polymerases in accordance with standard procedure. The transcripts are suspended in water at a final concentration of 0.2 mg/ml. Ovarian lobes are removed from adult female toads, Stage V, defolliculated

oocytes are obtained, and RNA transcripts (10 ng/oocyte) are injected in a 50 nl bolus using a microinjection apparatus. Two electrode voltage clamps are used to measure the currents from individual *Xenopus* oocytes in response to agonist exposure. Recordings are made in Ca^{2+} free Barth's medium at room temperature. The *Xenopus* system can be used to
5 screen known ligands and tissue/cell extracts for activating ligands.

Example 5: Microphysiometric Assays

Activation of a wide variety of secondary messenger systems results in extrusion of small amounts of acid from a cell. The acid formed is largely as a result of the increased metabolic activity required to fuel the intracellular signaling process. The pH changes in the
10 media surrounding the cell are very small but are detectable by the CYTOSENSOR microphysiometer (Molecular Devices Ltd., Menlo Park, CA). The CYTOSENSOR is thus capable of detecting the activation of a receptor which is coupled to an energy utilizing intracellular signaling pathway such as the G-protein coupled receptor of the present invention.

15 Example 6: Extract/Cell Supernatant Screening

A large number of mammalian receptors exist for which there remains, as yet, no cognate activating ligand (agonist). Thus, active ligands for these receptors may not be included within the ligand banks as identified to date. Accordingly, the 7TM receptor of the invention is also functionally screened (using calcium, cAMP, microphysiometer, oocyte
20 electrophysiology, etc., functional screens) against tissue extracts to identify natural ligands. Extracts that produce positive functional responses can be sequentially subfractionated until an activating ligand is isolated and identified.

Example 7: Calcium and cAMP Functional Assays

7TM receptors which are expressed in HEK 293 cells have been shown to be
25 coupled functionally to activation of PLC and calcium mobilization and/or cAMP stimulation or inhibition. Basal calcium levels in the HEK 293 cells in receptor-transfected or vector control cells were observed to be in the normal, 100 nM to 200 nM, range. HEK 293 cells expressing recombinant receptors are loaded with fura 2 and in a single day > 150

the stimulation or inhibition of cAMP production using standard cAMP quantitation assays. Agonists presenting a calcium transient or cAMP fluctuation are tested in vector control cells to determine if the response is unique to the transfected cells expressing the receptor.

Example 8: Characterization of the sequence

5 A purinergic-like receptor, designated as AXOR87 has been identified. The nucleotide and amino acid sequences of the receptor have been determined. These are set out below in SEQ ID NOs: 1 and 2. Suitable primers and probes were designed and used to analyse tissue expression. AXOR87 was found to be primarily expressed in mammary gland, parotid gland and thymus. The receptor is also expressed in myometrium, ovary,
10 lung, trachea, tonsil, skin, omentum, prostate, rectum and thyroid (Figure 1). AXOR87 mRNA expression was upregulated in all colon tumors versus normal colon. The receptor was down-regulated in all lung tumors versus normal tissue. Differential regulation was seen in breast tumors (Figure 2). AXOR87 mRNA expression was up-regulated in osteoarthritic knee and down-regulated in differentiated osteoblasts.
15 AXOR87 was expressed in dendritic and T cells and expression was up-regulated in growth factor-stimulated bone marrow cells. AXOR87 expression was upregulated in vascular endothelial growth factor-treated cells. (Figure 3).

Example 9: Chromosomal Localization

The chromosomal localization was also mapped. Human AXOR87 has been
20 mapped to 12q24, close to an asthma locus at 12q21-24, thus, AXOR87 may be a target for treatment of asthma.

SEQUENCE INFORMATION

SEQ ID NO:1

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 20 CACATTGTTGAGTGGCACTGA

SEQ ID NO:2

1 MYNGSCCRIE GDTISQVMPP LLIVAFVLGA LGNGVALCGF CFHMKTWKPS
 51 TVYLFNLAVA DFLLMICLPF RTDYLLRRRH WAFGDIPCRV GLFTLAMNRA
 101 GSIVFLTVVA ADRYFKVVHP HHAVNTISTR VAAGIVCTLW ALVILGTVYL
 25 151 LLENHLCVQE TAVSCSFIM ESANGWHDIM FQLEFFMPLG IILFCSFKIV
 201 WSLRRRQQLA RQARMKKATR FIMVVAIVFI TCYLPSVSAR LYFLWTVPS
 251 ACDPSVHGAL HITLSFTYMN SMLDPLVYYF SSPSFPKFYN KLKICSLKPK
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GP70703 sequence

SEQUENCE LISTING

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SmithKline Beecham PLC Glaxo Group Limited

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<140> TO BE ASSIGNED

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<151> 2000-05-30

<150> GB 0026839.1

<151> 2000-11-02

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GP70703 sequence

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<213> HOMO SAPIENS

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			20					25					30		
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		35					40					45			
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	50					55					60				
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GP70703 sequence

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Arg	Ser	Cys	Ile	Ser	Val	Ala	Asn	Ser	Phe	Gln	Ser	Gln	Ser	Asp	Gly
				325					330					335	
Gln	Trp	Asp	Pro	His	Ile	Val	Glu	Trp	His						
			340					345							

Claims

1. An isolated polypeptide selected from the group consisting of:
 - (a) an isolated polypeptide encoded by a polynucleotide comprising the sequence of SEQ ID NO:1;
 - (b) an isolated polypeptide comprising a polypeptide sequence having at least 95% identity to the polypeptide sequence of SEQ ID NO:2;
 - (c) an isolated polypeptide having at least 95% identity to the polypeptide sequence of SEQ ID NO:2; and
 - (d) fragments and variants of such polypeptides in (a) to (c).
2. The isolated polypeptide as claimed in claim 1 comprising the polypeptide sequence of SEQ ID NO:2.
3. The isolated polypeptide as claimed in claim 1 which is the polypeptide sequence of SEQ ID NO:2.
4. An isolated polynucleotide selected from the group consisting of:
 - (a) an isolated polynucleotide comprising a polynucleotide sequence having at least 95% identity to the polynucleotide sequence of SEQ ID NO:1;
 - (b) an isolated polynucleotide having at least 95% identity to the polynucleotide of SEQ ID NO:1;
 - (c) an isolated polynucleotide comprising a polynucleotide sequence encoding a polypeptide sequence having at least 95% identity to the polypeptide sequence of SEQ ID NO:2;
 - (d) an isolated polynucleotide having a polynucleotide sequence encoding a polypeptide sequence having at least 95% identity to the polypeptide sequence of SEQ ID NO:2;
 - (e) an isolated polynucleotide with a nucleotide sequence of at least 100 nucleotides obtained by screening a library under stringent hybridization conditions with a labeled probe having the sequence of SEQ ID NO: 1 or a fragment thereof having at least 15 nucleotides;
 - (f) a polynucleotide which is the RNA equivalent of a polynucleotide of (a) to (e); or a polynucleotide sequence complementary to said isolated polynucleotide

and polynucleotides that are variants and fragments of the above mentioned polynucleotides or that are complementary to above mentioned polynucleotides, over the entire length thereof.

- 5 5. An isolated polynucleotide as claimed in claim 4 selected from the group consisting of:
- (a) an isolated polynucleotide comprising the polynucleotide of SEQ ID NO:1;
 - (b) the isolated polynucleotide of SEQ ID NO:1;
 - (c) an isolated polynucleotide comprising a polynucleotide sequence encoding the polypeptide of SEQ ID NO:2; and
 - 10 (d) an isolated polynucleotide encoding the polypeptide of SEQ ID NO:2.
6. An expression system comprising a polynucleotide capable of producing a polypeptide of claim 1 when said expression vector is present in a compatible host cell.
- 15 7. A recombinant host cell comprising the expression vector of claim 6 or a membrane thereof expressing the polypeptide of claim 1.
8. A process for producing a polypeptide of claim 1 comprising the step of culturing a host cell as defined in claim 7 under conditions sufficient for the production of said
- 20 polypeptide and recovering the polypeptide from the culture medium.
9. An antibody immunospecific for the polypeptide of any one of claims 1 to 3.
10. A method for screening to identify compounds that stimulate or inhibit the function or
- 25 level of the polypeptide of claim 1 comprising a method selected from the group consisting of:
- (a) measuring or, detecting, quantitatively or qualitatively, the binding of a candidate compound to the polypeptide (or to the cells or membranes expressing the polypeptide) or a fusion protein thereof by means of a label directly or indirectly associated with the
 - 30 candidate compound;
 - (b) measuring the competition of binding of a candidate compound to the polypeptide (or to the cells or membranes expressing the polypeptide) or a fusion protein thereof in the presence of a labeled competitor.

- (c) testing whether the candidate compound results in a signal generated by activation or inhibition of the polypeptide, using detection systems appropriate to the cells or cell membranes expressing the polypeptide;
- (d) mixing a candidate compound with a solution containing a polypeptide of claim 1, to
- 5 form a mixture, measuring activity of the polypeptide in the mixture, and comparing the activity of the mixture to a control mixture which contains no candidate compound; or
- (e) detecting the effect of a candidate compound on the production of mRNA encoding said polypeptide or said polypeptide in cells, using for instance, an ELISA assay.



INVESTOR IN PEOPLE

Application No: GB 0112860.2
Claims searched: 1-10

Examiner: Dr Jeremy Kaye
Date of search: 11 December 2001

Patents Act 1977
Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.S):

Int Cl (Ed.7):

Other: Online: EPODOC, WPI, PAJ, BIOSIS, CAPLUS, EMBASE, GENBANK, MEDLINE, SCISEARCH

Documents considered to be relevant:

Category	Identity of document and relevant passage	Relevant to claims
E, X	WO 01/77320 A2 (BAYER) p.3, l.22 - p.6, l.20; p.16, l.23 - p.29, l.8; p.32, l.29 - p.41, l.27; Figures 1 & 2.	1-10
E, X	WO 01/73029 A2 (PE CORPORATION) p.14, l.16 - p.51, l.6; p.52-53	1-10

42

with one or more other documents of same category

earliest publication date of this invention

1. Patent document published on or after, but with priority date earlier than, the filing date of this application.

& Member of the same patent family